

CLAIMS

1. A method of simultaneously isolating both nucleic acids (RNA and DNA) from the same sample weighing not less than 0.5 mg - fresh, frozen, fixed or autoptic - including the following steps:
  - a) digestion of a sample incubated in a lysing solution consisting of: a caotropic agent, a ionic  
5 detergent, a proteolytic enzyme and a reducing agent;
  - b) enzyme inactivation by using extraction with a mixture of aromatic alcohols and producing an organic phase, that was stored and added to a second organic phase;
  - c) precipitation of RNA by adding a precipitating agent to the aqueous phase and an aliphatic short chain alcohol;
  - 10 d) precipitation of DNA from the organic phase as reported in step b) by using a precipitating agent and a short chain aliphatic alcohol.
2. Method in agreement with Claim 1 where the lysing solution used in step a) includes:
  - a caotropic agent, either urea or guanidine thiocyanate;
  - a ionic detergent, either SDS or SLS;
  - 15 - a proteolytic enzyme: proteinase K, trypsin, chymotrypin, pepsin or pronase;
  - a reducing agent, either  $\beta$ -mercaptoethanol or dithiotreitol.
3. Method in agreement with Claim 1 including also the addition of RNase inhibitor in step a), b), or c) of the method, alternatively.
4. Method in agreement with Claim 3 where the inhibitor is a Vanadyl ribonucleoside complex.
- 20 5. Method in agreement with Claims 1-4 including a nucleic acid precipitating agent, either tRNA or glycogen: alternatively added in step c) or step a) and also in step d) of the method
6. Method in agreement with Claim 5 wherein the precipitating agent is glycogen.
7. Method in agreement with Claim 6 where the final concentration of glycogen is no less than 10 ng/ml.
- 25 8. Method in agreement with Claim 7 wherein the final concentration of glycogen is no less than 50 ng/ml.
9. Method in agreement with Claims 1-8 wherein the short chain aliphatic alcohol is isopropanol or ethanol.
10. Method in agreement with Claim 2 wherein the guanidine salt in lysing solution of step a) is selected  
30 from the group consisting of guanidine thiocyanate and guanidine hydrochloride using a concentration ranging from 1 to 4 M.
11. Method in agreement with Claim 2 wherein the proteolytic enzyme in the lysing solution of step a) is proteinase K.
12. Method in agreement with Claim 11 wherein the concentration of proteinase K ranges from 0.1 to 10  
35 mg/ml and wherein the incubation with this enzyme is performed at a temperature more than 20°C.
13. Method in agreement with Claims 1 and 11-12 including at the end of step a) of the method a supplementary addition of the proteolytic enzyme with subsequent incubation.

14. Method in agreement with Claim 1 wherein the miscela of organic solvent and aromatic alcohol of step b) is phenol or a phenol/chloroform solution at acid pH, mainly between 5 and 6, and more preferably 5.5.
15. Method in agreement with Claim 14 wherein the volume ratio of phenol and chloroform is from 3:1 to 7:1 in the solution.
16. Method in agreement with Claims 1-15 wherein the aqueous phase is re-extracted with chlorophorm after the first extraction with aromatic alcohol following step b) of the method.
17. Method in agreement with Claims 1-16 wherein the excess salt is removed from the RNA precipitate obtained at step c) washing the pellet with a short chain alcohol diluted with deionised water.
18. Method in agreement with Claims 1 and 16, 17 where the aqueous solution at step b) and the deionised water are treated with DEPC.
19. Method in agreement with Claim 1 where the aliphatic alcohol added to precipitate DNA in agreement with step d) of the method is isopropanol and the precipitation is performed incubating the sample at a temperature lower than 0°C.
20. Method in agreement with Claim 19 wherein precipitated DNA is washed with a saline solution including at least 5% of organic solvent and wherein this step is optionally repeated to remove traces of phenol from precipitated DNA.
21. Method in agreement with Claim 20 wherein the saline solution is either citrate or Na Cl.
22. Method in agreement with Claim 21 wherein the solution is Na citrate at a concentration between 10 and 200 mM with pH from 6.8 to 7.3.
23. Method in agreement with Claims 1-22 wherein the biological material is represented by cell culture, tissue biopsy, tissue fragment or optionally by paraffin-embedded sections.
24. Method in agreement with Claim 23 wherein the paraffin-embedded sections are first deparaffinised using an organic solvent
25. Method in agreement with Claim 24 wherein the organic solvent is either xylene or benzene-derived.
26. Method in agreement with Claim 23 for extraction of viral nucleic acids from biological materials.
27. Method in agreement with Claim 23 wherein the nucleic acid is RNA.
28. Method in agreement with Claims 1-25 wherein the sample weighs no more than 20 mg and wherein the volume of lysing solution, in agreement with step a) is from 100 to 800 µl.
29. Kit for simultaneous and separate extraction of RNA and DNA from fresh and fixed samples, optionally also paraffin-embedded, in agreement with the method following Claims 1-28, including a tube with a lysing solution, a tube with a precipitating agent, a tube with a RNase inhibitor and instructions describing the method in agreement with Claims 1-28 and optionally sterile and RNase free tubes, disposable knives and alumina.
30. Kit for extraction of viral nucleic acids from fresh, fixed or autoptic biological samples, optionally also paraffin-embedded, in agreement with the method following Claims 1-28 including one or more tubes with oligonucleotides specific for viral identification using PCR, instructions describing the

method in agreement with Claims 1-28 and optionally tubes with reagents for retro transcription of RNA.